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Dye-penetration assay in embryo, third instar larva, and adult flies

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An abbreviated version of this protocol was published in eLIFE in Aug 2021

The cAMP effector PKA mediates Moody GPCR signaling in *Drosophila* blood–brain barrier formation and maturation

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Detailed protocol

- For the dye-penetration assay in **Embryo**, fluorescent dye (Texas red-coupled dextrane, 10 kDa, 2.5 mM; Molecular Probes) was injected from posterior into the body cavity of 21–22 hr embryos; after 10 min, dye diffusion was analyzed using confocal microscopy. Dye penetration was quantified by calculating the percentage of embryos showing visible dye penetration and as the mean pixel intensity (ranging from 0 to 255) within a representative window of the ventral portion of the nerve cord (n = 31–52). To adjust for variability in laser intensity, autofluorescent Convallaria was used for calibration. In addition, background as measured by mean pixel intensity in embryos without dye penetration was subtracted from the mean pixel intensities for all embryos processed in a batch. To assess significance, one-way ANOVA was performed over all groups with Student-Newman-Keuls post hoc test; for the rescue experiments, the $\chi 2$ test was used.
- For the dye penetration assay in **third instar larvae**, a fluorescent dye (Texas red-coupled dextran, 10 kDa, 10mg/ml, Molecular Probes) was injected into the body cavity of third instar larva. After 2.5 h, the cephalic complex was dissected, and the dye penetrated into the nerve cord was analyzed using Zeiss LSM710 confocal microscopy. Dye penetration was quantified by calculating the percentage of larva showing dye penetration and by measuring the mean pixel intensity within a representative window of the ventral portion of the nerve cord using Fiji software and normalized by dividing by the mean of the WT control group. To assess the significance of effects for the embryonic and larval dye penetration assays, Brown-Forsythe and Welch's ANOVA with multiple comparisons test was performed.
- The dye penetration assay in **adult flies** was performed as described in Bainton et al. (2005) with some critical modifications. Briefly, adult flies were hemolymph injected with 10mg/ml 10kDa Texas red-coupled dextran. After 2h, the injected flies were decapitated and their heads were mounted in a fluorinated grease covered glass slides with two compound eyes on the side (the proboscis facing up). Images were acquired on a Zeiss LSM710 confocal microscope at 200-300 µm depths from the eye surface with a Plan Fluor 10xw objective. Dye penetration was quantified by measuring the mean pixel intensities within a representative window of the central region of retina (n=18-30) of maximum-intensity Z projection of each image stack (z-section thickness 0.6 µm) by Fiji software, and normalized by the WT control. Statistical significance was assessed using the two-tailed unpaired t-test.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Li, X. and Steller, H. (2021). Dye-penetration assay in embryo, third instar larva, and adult flies. Bio-protocol Preprint. bio-protocol.org/prep1461.
2. Li, X., Fetter, R., Schwabe, T., Jung, C., Liu, L., Steller, H. and Gaul, U.(2021). The cAMP effector PKA mediates Moody GPCR signaling in *Drosophila* blood–brain barrier formation and maturation. eLIFE. DOI: 10.7554/eLife.68275